

# Dissolved Oxygen Protocol



Welcome

Introduction

Protocols

Learning Activities

Appendix

## **Purpose**

To measure the amount of oxygen dissolved in the water

## **Overview**

Students will use a dissolved oxygen kit to measure the dissolved oxygen in the water at their hydrology site. The exact procedure depends on the instructions in the dissolved oxygen kit used.

## **Student Outcomes**

Students will learn how to use the dissolved oxygen kit to collect data accurately and precisely, explain why the dissolved oxygen measurement is important, hypothesize about reasons for changes in the dissolved oxygen, and provide parameters for interpretation of dissolved oxygen data.

## **Science Concepts**

### *Physical Sciences*

Substances have characteristic properties.

### *Earth and Space Science*

Water is a solvent.

### *Life Sciences*

Organisms can only survive in environments where their needs are met.

Earth has many different environments that support different combinations of organisms.

Organisms change the environment in which they live.

Humans can change natural environments.

All organisms must be able to obtain and use resources while living in a constantly changing environment.

## **Scientific Inquiry Abilities**

Use a chemical test kit to measure dissolved oxygen.

Identify answerable questions.

Design and conduct scientific investigations.

Use appropriate mathematics to analyze data.

Develop descriptions and explanations using evidence.

Recognize and analyze alternative explanations.

Communicate procedures and explanations.

## **Time**

20 minutes

20 minutes for quality control procedure

## **Level**

Middle and Secondary

## **Frequency**

Weekly

Quality control procedure every 6 months

## **Materials and Tools**

*Hydrology Investigation Data Sheet*

Dissolved oxygen kit

Latex gloves

Safety goggles

Waste bottle with cap

Distilled water

For Quality Control Procedure:

100-mL graduated cylinder

250-mL polyethylene bottle with lid

Clock or watch

Thermometer

*Solubility of Oxygen Table*

*Correction for Elevation Table*

*Hydrology Investigation Quality Control*

*Procedure Data Sheet*

## **Preparation**

Suggested activity: *Practicing Your Protocols: Dissolved Oxygen*

## **Prerequisites**

Discussion of safety procedures when using chemical test kits



## Dissolved Oxygen – Introduction

Dissolved oxygen is the amount of molecular oxygen ( $O_2$ ) dissolved in water. It is not a measurement of the oxygen in the water molecule ( $H_2O$ ). Animals that live in the water need molecular oxygen dissolved in the water to breathe. The amount of dissolved oxygen in the water determines what can live there. Some animals, like salmon or mayfly larvae, require higher oxygen levels than other animals like catfish or leeches.



We call the maximum amount of dissolved oxygen the water will hold (under specific conditions) the solubility of dissolved oxygen. Factors affecting the solubility of dissolved oxygen include water temperature, atmospheric pressure, and salinity.



Cold water can hold more dissolved oxygen than warm water. For example, at  $25^\circ C$ , dissolved oxygen solubility is 8.3 mg/L, whereas at  $4^\circ C$  the solubility is 13.1 mg/L. Water can hold less dissolved oxygen at higher elevations because there is less pressure. Solubility of dissolved oxygen also decreases as salinity increases.



Dissolved oxygen can be added to water by plants during photosynthesis, through diffusion from the atmosphere, or by aeration. Aeration occurs when water is mixed with air. Such mixing occurs in waves and ripples.

The amount of dissolved oxygen is also affected by what lives in the water. Just as photosynthesis by terrestrial plants adds oxygen to the air we breathe, photosynthesis by aquatic plants contributes dissolved oxygen to the water. Water may become supersaturated, meaning that the dissolved oxygen levels are greater than its solubility. The extra dissolved oxygen would then eventually be released back into the air or be removed through respiration.



The living biota of water systems makes up only a very small portion of the total organic matter of the system. Most organic matter in aquatic ecosystems is non-living and it is collectively referred to as detritus. The organic matter can be produced *in situ* or enter water bodies from the surrounding land (from both natural and human

sources). The cycling of organic carbon between living and nonliving components is known as the carbon cycle. Organic matter is produced during photosynthesis and is consumed during respiration. During respiration, biota (fish, bacteria, etc.) consume dissolved oxygen.

We tend to find low dissolved oxygen levels, well under half the saturated value, in slow-moving streams near sources of organic matter and the surface of productive lakes during warm summer months.

## Teacher Support

The amount of oxygen dissolved in a body of water limits what can live in the water. Students often confuse the oxygen that is part of the water molecule (the O in  $H_2O$ ) with dissolved oxygen ( $O_2$ ). Dissolved oxygen is a natural impurity in water similar to dissolved solids. It is the dissolved oxygen that aquatic life needs for respiration. This is what we measure in the GLOBE *Dissolved Oxygen Protocol*. There is a lot more oxygen available in the atmosphere for animal respiration than in water. Roughly, two out of ten air molecules are molecular oxygen. In water, however, there are only five or six oxygen molecules for every million water molecules.

Dissolved oxygen test kits involve two parts - sample preservation (stabilization or fixing) and sample testing. Preservation involves the addition of a chemical to the sample that precipitates in the presence of dissolved oxygen, followed by the addition of a chemical that produces a colored solution. Testing involves adding drops of a titrant solution until the color disappears. The dissolved oxygen value is calculated from the volume of titrant added.

In the field, if time does not permit to have three students measure the dissolved oxygen of three different samples, have a student perform the whole measurement. Then have the other two students use the same fixed sample for sample testing later.

## Supporting Measurements

Oxygen solubility is dependent on temperature. It is therefore important to collect water temperature data along with dissolved oxygen data.



Atmosphere measurements such as cloud cover, precipitation, and air temperature may also be useful in interpreting dissolved oxygen data. Increased cloud cover, for instance, may result in a decrease in photosynthesis during the day.

It is also useful for hydrology measurements to know about the land cover in your watershed.

### **Student Preparation**

Students can make a standard to test their dissolved oxygen kits and method by shaking a partially filled bottle of distilled water for 5 minutes. This saturates the water with oxygen. Students should do the quality control procedure as described in the *Quality Control Procedure for Dissolved Oxygen Lab Guide* to test both the accuracy of their procedure and the precision of the kits. Doing the quality control will give students, teachers, and scientists confidence that the tests are being done properly.

### **Helpful Hints**

- Students should wear gloves when handling chemicals and water that may contain potentially harmful substances such as bacteria or industrial waste.
- Students should wear goggles when working with chemicals.
- Local authorities should be consulted on the proper disposal of used chemicals.
- Mark each item in the kit with a dot of paint or nail polish of the same color. Mark other kits with different colors to avoid having chemicals or titrators exchanged between kits.
- The amount of dissolved oxygen in the water can change rapidly once the sample has been collected. It is therefore important to do this test soon after the sample is collected. The water sample for the dissolved oxygen test should be ‘fixed’ at the water site (see instructions in your dissolved oxygen kit). Once the sample is fixed, the sample may be taken back to the school to finish the tests.
- Hold bottles and droppers vertically when adding drops of reagent to your water sample.

- If students are asked to ‘mix’, they should cap the bottle and do a ‘windshield wiper motion’ to gently mix the chemicals.
- The precipitate is settled when there is a distinct line between the clear liquid at the top and the settled material at the bottom (freshwater). It takes a long time (>15 minutes) for the precipitate to settle in salty and brackish water. Wait until there is a distinct line between clear liquid and settled material in the lower half of the bottle.
- Make sure you have no air bubbles in your titrator when you fill it.
- If your kit asks you to titrate to a “pale yellow”, hold a sheet of white paper behind the bottle and continue titration until the liquid is almost clear before adding the starch solution.

### **Instrument Maintenance**

1. Chemicals should be tightly capped immediately after they are used.
2. Rinse the sample bottle and titration tube with distilled water after use.
3. Discard chemicals from the dropper or titrator. They should not be put back into the original containers because they may be contaminated.
4. Do not rinse the titrator with distilled water as long as it has not been contaminated. Rinsing with distilled water often leaves a drop of water in the titrator that is difficult to remove.
5. Store the titrator with the plunger removed to avoid the rubber end sticking in the tube.

### **Questions for Further Investigation**

How would a change in the amount of dissolved oxygen affect what lives in a water body?

How could warming or cooling of the atmosphere affect the amount of dissolved oxygen in your water?

How could changes in the land cover around your water site affect the amount of dissolved oxygen in your water?

# Quality Control Procedure for Dissolved Oxygen

## Lab Guide

### **Task**

Check the accuracy of your dissolved oxygen kit. Practice using your dissolved oxygen kit properly.

### **What You Need**

- |  |  |
|--|--|
| <input type="checkbox"/> Dissolved oxygen test kit                           | <input type="checkbox"/> Your elevation (meters)                                   |
| <input type="checkbox"/> Distilled water                                     | <input type="checkbox"/> Latex gloves  |
| <input type="checkbox"/> 100-mL graduated cylinder                           | <input type="checkbox"/> Goggles   |
| <input type="checkbox"/> 250-mL polyethylene bottle with lid                 | <input type="checkbox"/> <i>Hydrology Investigation Quality Control Data Sheet</i> |
| <input type="checkbox"/> Clock or watch                                      | <input type="checkbox"/> GLOBE Science Log   |
| <input type="checkbox"/> Thermometer   | <input type="checkbox"/> Pen or pencil   |
| <input type="checkbox"/> Waste bottle with cap for discarding used chemicals |  |

### **What To Do**

1. Rinse the 250-mL bottle twice with distilled water.
2. Pour 100 mL of distilled water into the 250-mL bottle.
3. Put the lid on the bottle. Shake the bottle vigorously for 5 minutes. This is the standard you will use to test your kit.
4. Uncap the bottle and take the temperature of the water (see *Water Temperature Protocol Field Guide*). Be sure the tip of the thermometer does not touch the bottom or sides of the bottle.
5. Record the temperature of the distilled water standard on the *Hydrology Investigation Quality Control Data Sheet*.
6. Pour the standard into the sample bottle in your dissolved oxygen kit. Fill the sample bottle completely to the top. Put the lid on the sample bottle. Turn the bottle upside down while it is capped. There should not be any air bubbles.

**Note:** It is not necessary to immerse the sample bottle in the water to collect your sample when you are doing the quality control procedure.

7. Put on your gloves and protective goggles.
8. Follow the directions in your dissolved oxygen kit to measure the dissolved oxygen of your standard.
9. Record the amount of dissolved oxygen (mg/L) in your standard on your *Hydrology Investigation Quality Control Data Sheet*.
10. Look up the temperature you recorded earlier on the *Solubility of Oxygen Table*. See Table HY-DO-1.
11. Record the solubility for your water temperature.
12. Find the elevation closest to yours on the *Correction for Elevation/Pressure Table*. See Table HY-DO-2.
13. Record the correction value for your elevation.
14. Multiply the solubility of your standard times the correction value. This is the expected amount of dissolved oxygen in your standard.
15. Compare the amount of dissolved oxygen you measured with the kit to the expected amount for your standard.
16. If the measurement is within  $\pm 1\text{mg/L}$ , record the dissolved oxygen value on the *Hydrology Investigation Quality Control Procedure Data Sheet*. If the measurement is not within this range, repeat the entire quality control procedure.
17. If your measurements are still not in range, record the value you got and report to your teacher that the kit is not working properly.
18. Pour all used chemicals into the waste bottle. Clean your kit properly.

*Table HY-DO-1: Solubility of Oxygen in Fresh Water Exposed to Air at 760 mm Hg Pressure*

Temp °C	Solubility mg/L	Temp °C	Solubility mg/L	Temp °C	Solubility mg/L
0	14.6	16	9.9	32	7.3
1	14.2	17	9.7	33	7.2
2	13.8	18	9.5	34	7.1
3	13.5	19	9.3	35	7.0
4	13.1	20	9.1	36	6.8
5	12.8	21	8.9	37	6.7
6	12.5	22	8.7	38	6.6
7	12.1	23	8.6	39	6.5
8	11.9	24	8.4	40	6.4
9	11.6	25	8.3	41	6.3
10	11.3	26	8.1	42	6.2
11	11.0	27	8.0	43	6.1
12	10.8	28	7.8	44	6.0
13	10.5	29	7.7	45	5.9
14	10.3	30	7.6	46	5.8
15	10.1	31	7.4	47	5.7

*Table HY-DO-2: Correction Values For Various Atmospheric Pressures and Elevations*

Pressure millibars	elev m	Correction value %	Pressure millibars	elev m	Correction value %
1023	-84	1.01	841	1544	0.83
1013	0	1.00	831	1643	0.82
1003	85	0.99	821	1743	0.81
993	170	0.98	811	1843	0.80
988	256	0.97	800	1945	0.79
973	343	0.96	790	2047	0.78
963	431	0.95	780	2151	0.77
952	519	0.94	770	2256	0.76
942	608	0.93	760	2362	0.75
932	698	0.92	750	2469	0.74
922	789	0.91	740	2577	0.73
912	880	0.90	730	2687	0.72
902	972	0.89	719	2797	0.71
892	1066	0.88	709	2909	0.70
882	1160	0.87	699	3203	0.69
871	1254	0.86	689	3137	0.68
861	1350	0.85	679	3253	0.67
851	1447	0.84	669	3371	0.66

## Frequently Asked Questions

### 1. Why does the amount of dissolved oxygen I measured not agree with the amount I calculated?

There are two reasons why these numbers may not match.

First, you may not have followed the instructions on your kit exactly or you may have made small errors in the procedure you used. Here are some trouble-shooting tips:

1. Make sure you do not have any air bubbles in your sample bottle or your titrator (for kits that use a titrator). To check for air bubbles in the sample bottle, turn the bottle upside down while it is capped and look for bubbles.
2. Measure accurately. If you are adding drops from a bottle, hold the bottle vertically so that all of the drops are the same size.
3. Allow all of the precipitate to settle. If you shake the bottle too hard before the precipitate settles, it may take 10 minutes or more for the settling to happen.
4. Record accurately. If your kit asks you to count drops, have two people count to insure accuracy. If your kit asks you to read a titrator, make sure to read the instructions for accurately reading the titrator that come with your kit.

The second reason your measured value may not be the same as your calculated value is that there may be something wrong with the chemicals in your kit. In this case, you will need to get new chemicals.

### 2. What is oxygen solubility?



Oxygen solubility is the amount of oxygen that can be dissolved in the water. Solubility varies with water temperature, amount of dissolved solids, and atmospheric pressure. Cold water is able to hold more dissolved oxygen. As temperatures go up, water releases some of its oxygen into the air. Solubility decreases with salinity and decreasing air pressure. We control these variables in our dissolved oxygen standard by using distilled water, and correcting for the water temperature and elevation (an indirect measure of air pressure).

### 3. The sampling procedure in the kit says to submerge the sample bottle in the water. How can I do this with my shaken standard?

The shaken standard can be poured directly into the sample bottle until the bottle is completely filled. You will not add oxygen to the sample by pouring it since the water sample is already saturated with oxygen. Once the sample bottle is filled, continue with the instructions for fixing the sample.

# Dissolved Oxygen Protocol

## Field Guide

### Task

Measure the dissolved oxygen of your water sample.

### What You Need

- |   |   |
|---|---|
| <input type="checkbox"/> Hydrology Investigation Data Sheet | <input type="checkbox"/> Distilled water                          |
| <input type="checkbox"/> Latex gloves                       | <input type="checkbox"/> Waste bottle with cap for used chemicals |
| <input type="checkbox"/> Goggles                            | <input type="checkbox"/> GLOBE Science Log                        |
| <input type="checkbox"/> Dissolved oxygen kit               | <input type="checkbox"/> Pen or pencil                            |

### In the Field

1. Fill in the top of the *Hydrology Investigation Data Sheet*.
2. Put on the gloves and goggles.
3. Rinse the sample bottle and your hands with sample water three times.
4. Place the cap on the empty sample bottle.
5. Submerge the sample bottle in the sample water.
6. Remove the cap and let the bottle fill with water. Move the bottle gently or tap it to get rid of air bubbles.
7. Put the cap on the bottle while it is still under the water.
8. Remove the sample bottle from the water. Turn the bottle upside down to check for air bubbles. If you see air bubbles, collect the sample again.
9. Follow the directions in your Dissolved Oxygen Kit to test your water sample.
10. Record the dissolved oxygen in your water sample on the *Field Sheet* as Observer 1.
11. Have two other students repeat the measurement using a new water sample each time.
12. Record their data on the *Field Sheet* as Observers 2 and 3.
13. Calculate the average of the three measurements.
14. Each of the three measurements should be within 1 mg/L of the average. If one of the measurements is not within 1 mg/L of the average, find the average of the other two measurements. If both of these measurements are within 1 mg/L of the new average, record this average.
15. Discard all used chemicals into the waste container. Clean your dissolved oxygen kit with distilled water.
16. Report the data to GLOBE and place your *Hydrology Investigation Data Sheet* in your *Data Book*



## Frequently Asked Questions

### 1. How much dissolved oxygen should I have in my water?

The amount of dissolved oxygen you will measure depends on your water site. Dissolved oxygen is added to water through aeration (water running or splashing) and by photosynthesis of aquatic plants. It is used up by respiration. The maximum amount of dissolved oxygen your water can hold (saturated solution) depends on elevation/pressure at your site, water temperature, and salinity of your sample. Warm, still waters might have dissolved oxygen levels of about 4 or 5 mg/L. Cold, running waters might have oxygen levels at 13 or 14 mg/L. Oxygen levels can vary throughout the day, with the maximum at peak sunlight (peak photosynthesis). Maximum levels can be supersaturated (above solubility).

### 2. Why do we have to do the measurements at the same time of day?

The amount of dissolved oxygen may change during the day as the water begins to warm up. More light penetrating the water causes more photosynthesis to occur. This can also increase the amount of dissolved oxygen. For this reason it is important to do your Hydrology measurements at the same time of day each week.

### 3. What will make my dissolved oxygen levels change over the year?



Besides seasonal differences in temperature, seasonal changes in the flow of your stream, changes in transparency, or changes in productivity (amount of growth of plants and animals in the water) will cause changes in dissolved oxygen levels.

### 4. When we perform the Quality Control Procedure on our standard, we are required to compensate for elevation at our location. Do we have to compensate for elevation when we are testing an actual water sample?

The compensation is only necessary to determine the theoretical saturated value for dissolved oxygen, which depends on elevation. You then compare the measured dissolved oxygen in your standard solution with the saturated value from the table (compensated for elevation) in order to determine if your kit and procedures are correct. There is no compensation required when measuring the actual amount of dissolved oxygen in a water sample from your Hydrology site.



## What do people look for in the data?

The amount of dissolved oxygen in a water body depends on many variables such as temperature, elevation, water flow, and amount of vegetation in the water. A warm pond may naturally have less dissolved oxygen than a cold stream. Most organisms will not exist at dissolved oxygen levels less than 3.0 mg/L. Some sensitive organisms will not live in oxygen levels less than 7.5 mg/L. Dissolved oxygen levels that drop at low levels (i.e., less than 5 mg/L) are a reason for concern. Excess nutrients (e.g., fertilizer, organic-rich waste water) added to the water body can cause an overgrowth of vegetation and algae, causing increased decay in the water.

In addition to looking at the amount of dissolved oxygen in the water, it is also interesting to compare the amount of measured dissolved oxygen with a calculated value for saturation. This can tell us about the productivity of the water body. In a productive water body, plants will be producing oxygen through photosynthesis. Dissolved oxygen values will vary throughout the day, with maximum value occurring in the early afternoon and lowest levels occurring during the night (when respiration is not balanced by photosynthesis). At certain times of the day (typically early afternoon), some water bodies may actually have a dissolved oxygen measurement above the saturation level, indicating that more oxygen is being produced by photosynthesis than is being consumed by respiration. Water bodies that are highly turbid have low light penetration and low productivity. They are typically characterized by low dissolved oxygen levels.

The GLOBE visualizations page on the Web site displays values of saturated dissolved oxygen for your site that you can compare graphically with your actual measurements.

## An Example of a Student Research Investigation

### Forming a Hypothesis

A student interested in dissolved oxygen is looking at the time plot of dissolved oxygen at Reynolds Jr Sr High School SWS-02 site, called “Covered Bridge” (Figure HY-DO-2). She notices that the values of dissolved oxygen in late December 2000

through January 2001 were much lower than values in previous winters. During that time period they ranged from 7 to 10 mg/L for about a month. During the previous three winters, dissolved oxygen consistently ranged from 11 to 15 mg/L. The low values are similar to those found during the warmer periods.

Knowing that the *saturated* dissolved oxygen levels are usually related to temperature, she hypothesizes that the *water temperature during this time period is higher than normal* and the *warmer water is responsible for the lower dissolved oxygen values*.

She contacts the school and learns that this water body is the Shenango River.

### Collecting and Analyzing Data

She begins by plotting the monthly mean values of dissolved oxygen and temperature. See Figure HY-DO-3.

The unusually low January 2001 dissolved oxygen is even more apparent when looking at the monthly averages. However, there does not appear to be a corresponding increase in water temperature, which is about 3° C.

If temperature is normal, then the values of saturated dissolved oxygen should be high as well. This would mean that the *dissolved oxygen deficit*, which is the difference between the saturated and observed values, is unusually high for some reason.

The GLOBE visualizations page will calculate monthly averages for water temperature and measured dissolved oxygen, but not for saturated dissolved oxygen, so the student decides to calculate the monthly averages for saturated dissolved oxygen herself. She generates a plot with dissolved oxygen, saturated dissolved oxygen, and water temperatures, and then creates a data table. She transfers this information into a spreadsheet.

She extracts all the January values for each of the years (Table HY-DO-3). She then calculates the dissolved oxygen deficit (saturated dissolve oxygen – measured dissolved oxygen). Then for each year, she calculates the average for each of the four terms.



The average dissolved oxygen in 2001 was 8.9 mg/L. In 1998-2000, it was 11.6, 11.8 and 13.2, respectively.



However, the water temperature was about the same for all four Januarys: 3.2°, 0.4°, 1.1° and 2.5° C. The temperature was actually warmer in January of 1998 than 2001, and the measured DO was higher. Therefore, the decrease in dissolved oxygen does not seem to be related to temperature



The average dissolved oxygen deficit ranged from 1.0 to 2.7 mg/L the first three years, and was 4.8 in 2001. The dissolved oxygen deficit is almost twice as high in January 2001 as it was in the next highest year (January 1999) when it was 2.7.

She concludes that: *Measured dissolved oxygen values are lower in January 2001 than in January 1998-2000. Water temperature and saturated dissolved oxygen values are about the same, so the decrease in dissolved oxygen is not related to a change in water temperature.*



Therefore her hypothesis that warmer water was causing the lower dissolved oxygen value was rejected. It is all right to disprove your hypothesis. Scientist do this all the time. Often in finding out that our hypothesis is not correct, we come up with alternatives that lead to a better understanding of the problem at hand.



### **Future Research**

There is nothing in this data to suggest WHY the dissolved oxygen is so much lower in winter 2001 than during the 3 previous years. The student does notice that the 2000-2001 winter seems longer in duration than the other winters but cannot think of why that might affect dissolved oxygen levels later in the winter. She also notices that the summer dissolved oxygen data in 2000 appear more variable than in previous years. Perhaps something else has changed in the river to cause a higher demand for dissolved oxygen. One reason might be that more bacteria, such as those associated with decaying organic matter from sewage, might be present in the water. A student might investigate whether there have been external changes in the watershed.



**Table HY-DO-3**

	Water Temp. degrees C	Dissolved oxygen mg/L	Saturated DO mg/L	DO use mg/L
Date				
1/2/1998	5	11.2	12.8	1.6
1/10/1998	5.5	10.5	12.6	2.1
1/17/1998	2	12.1	13.8	1.7
1/24/1998	1.5	12.6	14	1.4
1/31/1998	2	11.7	13.8	2.1
Average	3.2	11.6	13.4	1.8
Date				
1/9/1999	0	12.3	14.6	2.3
1/16/1999	0	12.3	14.6	2.3
1/23/1999	1	10.8	14.2	3.4
1/30/1999	0.5	11.6	14.4	2.8
Average	0.4	11.8	14.5	2.7
Date				
1/6/2000	3	13.6	13.5	-0.1
1/13/2000	1.2	13	14.1	1.1
1/20/2000	0	13	14.6	1.6
1/27/2000	0	13.3	14.6	1.3
Average	1.1	13.2	14.2	1.0
Date				
1/5/2001	6	9.8	12.4	2.6
1/12/2001	1	9.8	14.2	4.4
1/19/2001	2	8.5	13.8	5.3
1/26/2001	1	7.4	14.2	6.8
Average	2.5	8.9	13.7	4.8

Figure HY-DO-2

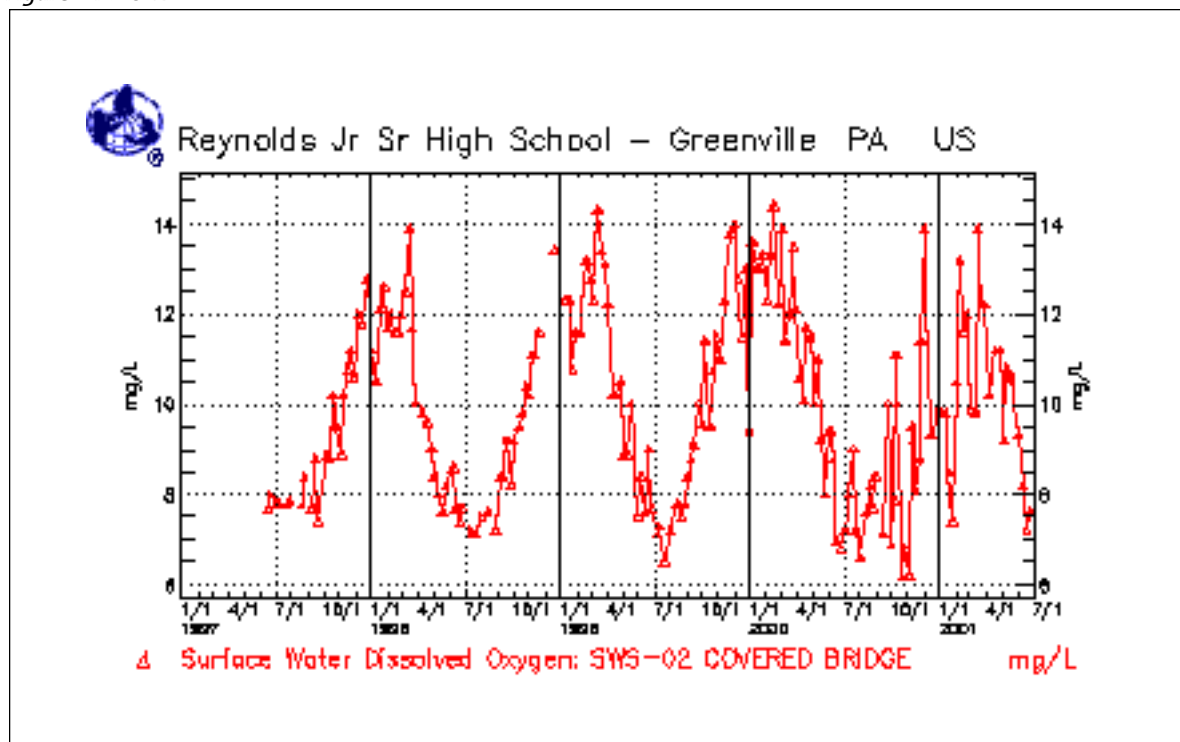


Figure HY-DO-3

